

## Rapid *Anopheles* vision mutation screening V 2



*Anopheles* mosquitoes undergo induced color change (called homochromy) based on perception of the background against which they are cultured.<sup>1</sup> When larvae are reared on either a dark black or white background, they become pigmented dark or pale respectively as shown by the pair of *Anopheles albimanus* larvae at left. The degree of darkening depends in part on the length of time the larvae have been cultured in a black container and the degree of fat body development. Therefore, larvae cultured during their entire development in a dark container at a low density show this change most dramatically. This color

change depends on the normal eye pigmentation and, presumably, on the proper function of any pigmentation and signaling pathways involved in the response.

The method simply requires culturing the larvae from at least the second stage in black or dark-colored containers that are illuminated. (Larvae cultured in darkness will develop typical pale pigmentation.) The source of illumination does not appear to be critical. Occasional transfers of a few minutes to white containers for feeding or correcting the density does not interfere with the effect.

At the L3 or L4 stage, larvae are scanned *en masse* in the dark tray in a well-illuminated location for those that appear lighter in color. They are usually quite apparent as demonstrated by the two larvae at the right, but purposely seeding a sample of dark larvae with a few that are pale will demonstrate the degree of effect that can be expected. These individuals are transferred to a dish for microscopic examination. Leaving the larvae undisturbed during examination provides better visualization since the dorsal side coloration is a more consistent indicator of general color. After this initial selection, it is also helpful to transfer the larvae to a white tray and scanning for pale individuals. Usually, no more than approximately 25 larvae per thousand cultured in this way require individual examination.



### References:

1. Benedict, M.Q. and H. Chang. 1996. Rapid isolation of anopheline mosquito eye-color mutants, based on larval color change. **Medical and Veterinary Entomology**. 10(1): 93-96.
2. Benedict, M.Q., N.J. Besansky, H. Chang, O. Mukabayire, and F.H. Collins. 1996. Mutations in the *Anopheles gambiae* *Pink-Eye* and *White* genes define distinct, tightly linked eye-color loci. **Journal of Heredity**. 87(1): 48-53.

<sup>1</sup> Before beginning a large screen, it is advisable to culture a thousand or so larvae of the species of choice in dark containers. While all individuals of most species change color, some laboratory stocks have a low frequency of individuals that do not.